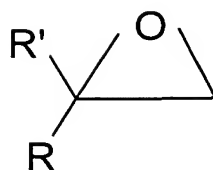


Claims

1. A process for the hydrolysis of a fluorinated epoxide comprising one or more CF<sub>3</sub> groups, in which process  
5 the epoxide is treated, in the presence of water, with a protein having an epoxide hydrolase (EH) activity on CF<sub>3</sub> epoxides so as to induce opening of the epoxide and formation of the vicinal diol.
- 10 2. The process as claimed in claim 1, in which the protein having an epoxide hydrolase (EH) activity on CF<sub>3</sub> epoxides comprises the following amino acid sequence:
  - 15 i. the amino acid sequence SEQ ID NO: 2; or
  - ii. a sequence having a percentage homology of greater than or equal to 40%, preferably greater than or equal to 80%, more preferably greater  
20 than or equal to 85%, even more preferably greater than or equal to 90%, and better still greater than or equal to 95, 96, 97, 98 or 99%, with SEQ ID NO: 2, the protein thus defined having an EH activity on CF<sub>3</sub> epoxides;
  - 25 iii. a sequence comprising at least 10, preferably at least 20, more preferably at least 50 or 100, consecutive amino acids of SEQ ID NO: 2 or of a sequence as defined in ii, the protein thus  
30 defined having an EH activity on CF<sub>3</sub> epoxides.
3. The process as claimed in claim 1 or 2, in which the  
35 protein is encoded by a nucleic acid comprising the following sequence:
  - (a) the nucleotide sequence represented in SEQ ID  
NO: 1;

- (b) a nucleotide sequence which encodes the amino acid sequence SEQ ID NO: 2;
- 5 (c) a nucleotide sequence which differs from the sequence according to (a) or (b) by virtue of the degeneracy of the code;
- 10 (d) a nucleotide sequence which hybridizes to a sequence according to (a), (b) or (c), and encoding a protein having an EH activity on CF<sub>3</sub> epoxides;
- 15 (e) a nucleotide sequence having a percentage identity of greater than or equal to 45%, preferably greater than or equal to 80%, more preferably greater than or equal to 85%, even more preferably greater than or equal to 90%, and even better still greater than or equal to 95, 96, 97, 98 or 99%, with SEQ ID NO: 1, and encoding a protein having an EH activity on CF<sub>3</sub> epoxides;
- 20 (f) a fragment of a nucleotide sequence according to (a), (b), (c), (d) or (e), comprising at least 30, preferably at least 60, more preferably at least 150 or 300, consecutive nucleotides, and encoding a protein having an EH activity on CF<sub>3</sub> epoxides.
- 25
- 30 4. The process as claimed in one of claims 1 to 3, in which the protein is the epoxide hydrolase of *Aspergillus niger* LCP521, which is natural or recombinant.
- 35 5. The process as claimed in any one of claims 1 to 4, characterized in that the epoxide corresponds to formula (I):



in which:

- 5        - the group R is an alkyl, alkenyl, cycloalkyl, aryl or aralkyl group optionally substituted with alkyl, alkoxy, alkylthio or halogen; R optionally comprising one or more hetero atoms such as O or S; the alkyl, alkoxy and alkylthio substituents comprising a linear, branched or  
10        cyclic C<sub>1</sub>-C<sub>6</sub>, preferably C<sub>1</sub>-C<sub>3</sub>, hydrocarbon-based chain, optionally comprising one or more halogen atoms, such as Cl, F or Br, preferably F;
- 15        - the group R' is H or a linear, branched or cyclic C<sub>1</sub>-C<sub>10</sub>, preferably C<sub>1</sub>, C<sub>2</sub> or C<sub>3</sub> alkyl, optionally comprising one or more hetero atoms, in particular halogen atoms, such as Cl, F or Br, preferably F, or else hetero atoms such as  
20        O or S;
- it being understood that at least one of the radicals R and R' is, or comprises, one or more, preferably from 1 to 3, trifluoromethyl (CF<sub>3</sub>) groups.
- 25        6. The process as claimed in claim 5, in which the epoxide of formula (I) is such that R' is H or a C<sub>1</sub>, C<sub>2</sub> or C<sub>3</sub> linear alkyl, preferably R' is H or C<sub>1</sub> alkyl optionally substituted with one or more  
30        halogen atoms, preferably with 3 F atoms.
7. The process as claimed in claim 5 or 6, in which, in the formula (I), the groups R are selected from the following groups:
- 35        - linear or branched alkyls comprising from 1 to 10 C, preferably from 1 to 6 C, optionally

- substituted with one or more halogen atoms, such as Cl, F or Br, preferably F;
- cycloalkyl comprising from 3 to 10 C, preferably from 3 to 8 C, optionally substituted with one or more halogen atoms, such as Cl, F or Br, preferably F;
  - phenyl or naphthyl, optionally substituted with one or more halogen atoms, such as Cl, F or Br, preferably F;
  - aralkyl comprising from 7 to 18 C, optionally substituted with one or more halogen atoms, such as Cl, F or Br, preferably F.
8. The process as claimed in one of claims 5 to 7, in which R comprises from 1 to 3 CF<sub>3</sub> groups.
9. The process as claimed in one of claims 5 to 8, in which R is a phenyl substituted with from 1 to 3 groups selected from trifluoromethyl, trifluoromethoxy and trifluoromethylthio.
10. The process as claimed in any one of claims 1 to 9, in which the epoxide is a mixture of (R) and (S) enantiomers.
11. The process as claimed in claim 10, in which the mixture is racemic.
12. The process as claimed in one of claims 1 to 12, in which an epoxide hydrolysis is carried out with an enantioselectivity coefficient of greater than or equal to 10, preferably of greater than or equal to 30.
13. The process as claimed in claim 10 or 11, in which an enantioselective hydrolysis is carried out and a mixture enriched in one of the isomers and in the diol corresponding to the other isomer is produced.

14. The process as claimed in one of claims 1 to 13,  
in which a preparation enriched in (S) epoxide and  
in (R) diol is produced.
- 5
15. The process as claimed in claim 14, in which, at  
the end of the hydrolysis reaction, the (R) diol  
is separated from the (S) epoxide, and the latter  
is recovered.
- 10
16. The process as claimed in one of claims 1 to 13,  
in which a preparation enriched in (R) epoxide and  
in (S) diol is produced.
- 15
17. The process as claimed in claim 16, in which, at  
the end of the hydrolysis reaction, the (S) diol  
is separated from the (R) epoxide, and the latter  
is recovered.
- 20
18. The process as claimed in one of claims 1 to 13,  
in which a preparation enriched in (R) diol is  
produced and, at the end of the hydrolysis  
reaction, the (S) epoxide is separated from the  
(R) diol, and the latter is recovered.
- 25
19. The process as claimed in one of claims 1 to 13,  
in which a preparation enriched in (S) diol is  
produced and, at the end of the hydrolysis  
reaction, the (R) epoxide is separated from the  
(S) diol, and the latter is recovered.
- 30
20. The process as claimed in one of claims 1 to 11  
and 13, in which an epoxide hydrolysis is carried  
out with an enantioselectivity coefficient of less  
than 10.
- 35
21. The process as claimed in claim 20, in which the  
(R) and (S) isomers are hydrolyzed and a racemic  
or nonracemic diol is produced.

22. The process as claimed in any one of claims 1 to 21, in which the epoxide is in solution in a water-miscible organic solvent.
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23. The process as claimed in claim 22, in which this solvent is selected from dimethyl sulfoxide, dimethylformamide, acetone, tetrahydrofuran, dioxane and propanol, and mixtures thereof.
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24. The process as claimed in any one of claims 1 to 21, in which the epoxide is in solution in a water-immiscible organic solvent.
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25. The process as claimed in claim 24, in which this solvent is selected from isooctane, hexane, cycloalkanes and aromatic compounds, and mixtures thereof.
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26. The process as claimed in claim 24 or 25, in which an emulsion is formed between the organic solution of the epoxide and an aqueous solution of the protein with EH activity.
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27. The process as claimed in any one of claims 1 to 26, in which the protein with EH activity is in an aqueous solution.
- 30
28. The process as claimed in any one of claims 1 to 12 and 14 to 20, for producing an enantiomeric excess of (R) or (S) epoxide of greater than or equal to 97%.
- 35
29. The process as claimed in any one of claims 1 to 19, in which a preparation that is enantiopure or enantiomerically enriched in (R) or (S) epoxide or diol is produced, the epoxide or the diol being a pharmaceutical, plant protection or agrochemical

product or an intermediate of a pharmaceutical, plant protection or agrochemical product.

- 5 30. A composition that is useful for implementing the process as claimed in any one of claims 1 to 29, comprising, for successive or simultaneous addition, a fluorinated epoxide comprising one or more  $\text{CF}_3$  groups and an organic solvent.
- 10 31. The composition as claimed in claim 30, comprising a water-miscible organic solvent selected from: dimethyl sulfoxide, dimethylformamide, acetone, tetrahydrofuran, dioxane and propanol, and mixtures thereof.
- 15 32. The composition as claimed in claim 30, comprising a water-immiscible organic solvent selected from: isooctane, hexane, cycloalkanes and aromatic compounds, and mixtures thereof.
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